IVD

For professional use only

HIV PCR detection Kit

(*PREP-NA* DNA/RNA Extraction Kit included)

USER MANUAL

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1. INTENDED USE

The **HIV PCR detection Kit** is intended for research and diagnostic applications. The **HIV PCR detection Kit** is an *in vitro* Nucleic Acid Test (NAT) based pathogen detection product. The **HIV PCR detection Kit** is designed to detect Human Immunodeficiency Virus type 1 (HIV-1) nucleic acids in human blood plasma.

The **HIV PCR detection Kit** can be used in clinical practice for HIV diagnostics.

2. METHOD

The implemented method is based on viral RNA reverse transcription followed by PCR amplification of the obtained cDNA.

The detection can be performed in each of two variants: real-time, and end-point fluorescent detection (FLASH technology).

The **HIV REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes bearing reporter and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided. The **HIV FLASH PCR Detection Kit** is based on the same principle but the fluorescence is measured only once after reaction.

The automatic analysis for **HIV REAL-TIME PCR Detection Kit** is available on “DNA-Technology” made instruments: DT*lite* and DT*prime* Thermal Cyclers (see the catalogue at www.dna-technology.ru/en to see available supply options) and for **HIV FLASH PCR Detection Kit** on Gene or Gene-4 Fluorescence Readers

O-GENE-EU, O-GENE4-EU.

The **HIV REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene (Qiagen) real-time thermal cyclers. The **HIV FLASH PCR Detection Kit** is also approved for use with Ala1/4 fluorescence reader (BioSan).

3. CONTENT

The Kit consists of three parts, namely: PREP-NA DNA/RNA Extraction Kit, Reverse Transcription Kit and HIV PCR detection Kit (either Real-time or FLASH variant) each is providing the crucial step of the assay.

Table 1. *PREP-NA* DNA/RNA Extraction Kit

Reagent	Description	Total volume	Amount
Lysis buffer	Colorless, soapy liquid	30 mL	1 vial
Precipitation buffer	Colorless liquid	40 mL	1 vial
Washout solution 1	Colorless liquid	50 mL	1 vial
Washout solution 2	Colorless liquid	30 mL	1 vial
Dissolving buffer	Colorless liquid	5 mL (1.25 mL in each tube)	4 tubes
Negative control (C-)	Colorless liquid	3 mL (1.5 mL in each tube)	2 tubes
Internal control (RNA-IC)	Colorless liquid	1 mL	1 tube

Table 2. Reverse Transcription Kit

Reagent	Description	Total volume	Amount
RT-buffer	Colorless liquid	200 µL	1 tube
RT-sum	Colorless liquid	100 µL	1 tube
Reverse transcriptase	Colorless liquid	50 µL	1 tubes

Table 3. HIV PCR detection Kit

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless liquid and white waxy fractions	1.92 mL or 2,0 mL (0.02 mL per tube)	96 or 100 separate or stripped tubes of 0,2 or 0,5 mL
TECHNO Taq-polymerase	Colorless viscous liquid	50 µL	1 tube
PCR-buffer	Colorless liquid	1 mL (0.5 mL in each tube)	2 tubes
Positive control (C+)	Colorless liquid	150 µL	1 tube
Mineral oil (not supplied in Kit for Rotor-Gene)	Colorless viscous liquid	2 mL (1 mL in each tube)	2 tubes
Background buffer[1]	Colorless liquid	0.2 mL	1 tube

[1] Applicable to FLASH PCR kits

The approximate total time needed to perform the assay is 5 hours.

The *PREP-NA* DNA/RNA Extraction Kit is sufficient for extraction of 100 samples.

The **HIV PCR detection Kit** sufficient to test 96/100 samples including IC, negative and positive control samples.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1 **Specimen collection**

The blood samples should be collected in 2 or 4 mL Vacuette type tubes with EDTA in 2.0 mg/mL final concentration. The sodium citrate anticoagulant is also applicable.

The use of heparin anticoagulant is not allowed.

4.2 **RNA extraction and PCR**

Vortex mixer;

0.2, 0.5 and 1.5 mL tubes;

PCR tube rack for 0.2, 0.5 and 1.5 mL tubes;

Single channel pipettes (volume range 2-20 µl, 20-200 µl, 200-1000 µl);

RNase and DNase free filtered pipette tips (volume range 20 µl, 200 µl, 1000 µl);

Powder-free surgical gloves;

Disinfectant solution;

Container for used pipette tips;

High speed centrifuge (13000 rpm);

Thermostat (temperature range 50-95°C);

Real-time PCR thermal cycler (for **HIV REAL-TIME PCR Detection Kit**);

Tercyc Conventional PCR Thermal Cycler (REF O-TP4-EU) or equivalent (for **HIV FLASH PCR Detection Kit**);

Gene or Gene-4 Fluorescence Reader (REF O-GENE-EU, O-GENE4-EU) or Ala1⁄4 fluorescence reader or equivalent (for **HIV FLASH PCR Detection Kit**);

5. WARNINGS AND PRECAUTIONS

The laboratory makeup should comply the requirements regulating work with microorganisms of I-IV classes of pathogenicity.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapour/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Do not use the kit after the expiry date provided. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

6. RNA EXTRACTION PROTOCOL

The **HIV PCR detection Kit** is designed to detect DNA extracted from blood plasma. Shake the tube containing blood sample thoroughly to mix the blood and anticoagulant.

The overall storage of the sample should not exceed 6 hours.

The transportation and storage temperature from collecting the sample till analysis should be in 2-8 °C range.

6.1 To obtain the plasma spin the tubes with blood at 3000 rpm for 20 min at room temperature (18–25 °C).

6.2 Take the upper fraction (plasma) with an automatic sampler and put it into the new 1.5 mL tube. The blood plasma can be stored at minus20 °C for 3 months.

The lysis buffer can contain the precipitate. Dissolve it at 65 °C for 10 min. prior to use.

At this step of assay use only RNase and DNase free pipette tips.

To rise the reliability of the results it is advised to perform the extraction in duplicates.

6.3 Mark the required number of 1,5 mL tubes by the following scheme: for each test sample and for negative control (C-).

6.4 For example: if you need to test 10 samples, mark 11 tubes (10 for the samples, 1 for C-).

6.5 Add 10 µL of the premixed internal control (RNA-IC) in each tube.

6.6 Add 300 µl of the lysis buffer avoiding contact of the pipette tip with an edge of the tube. Close the tubes.

Open the tube, add sample, then close the tube before proceeding to the next DNA sample to prevent contamination.

6.7 Add 100 µL of the blood plasma sample into the marked tubes. Do not add samples to the “C-“ tube.

6.8 Add 100 µL of the “C-“ into corresponding tube.

6.9 Close the tubes and mix them for 3–5 s twice.

6.10 Incubate the tubes for 15 min at 65 °C, spin down the drops at 13000 rpm for 30 s at room temperature (18–25 °C).

6.11 Add 400 µl of the precipitation buffer into all tubes. Close the tubes and mix them for 3-5 s twice.

6.12 Spin the tubes at 13000 rpm for 15 min at room temperature (18–25 °C).

6.13 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.14 Add 500 µl of the washout solution №1 to the precipitate and shake the tube thoroughly.

6.15 Spin the tubes at 13000 rpm for 5 min at room temperature (18–25 °C).

6.16 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.17 Add 300 µl of the washout solution №2 to the precipitate and shake the tube thoroughly.

6.18 Spin the tubes at 13000 rpm for 5 min at room temperature (18–25 °C).

6.19 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.20 Open the tubes and dry the precipitate at 65 °C for 5 min.

6.21 Add 16.5 µL of the elution buffer to the precipitate. Spin down the drops for 3–5 s.

6.22 Incubate the tubes for 10 min at 65 °C.

6.23 Spin down the drops at 13000 rpm for 30 s.

7. REVERSE TRANSCRIPTION PROTOCOL

7.1 Thaw content of «RT-buffer» and «RT-mix» tubes from Reverse Transcription Reagent Set at room temperature, then vortex thoroughly and spin down drops by centrifuging at 1000-3000 RPM for 3-5 sec.

7.2 Prepare RT-mix by mixing together RT Buffer, RT-sum and reverse transcriptase in separate plastic tube:

- 2,0 x (N+1) µl of the RT-buffer,
- 1,0 x (N+1) µl of the RT-sum,
- 0,5 x (N+1) µl of the reverse transcriptase,

where N+1 – is the number of samples being analyzed, considering «C-» (N) and one extra sample.

CAUTION! Reverse transcriptase should be kept out of freezer chamber for as short time as possible.

7.3 Vortex RT-mix and spin down drops by centrifuging at 1000-3000 RPM for 3-5 sec.

7.4 Transfer tube with RT-mix to NA extraction room.

7.5 Add 3,5 µl of the RT-mix to each tube with isolated RNA sample and to «C-» tube. Pipette 5-7 times to mix the content of the tube.

7.6 Place tubes in thermostat and incubate at 40oC for 30 min, then heat up to 95oC and leave for 5 min.

7.7 Spin the tubes at 13000 RPM for 30 sec to collect the drops.

The cDNA preparation is ready for PCR.

Note. The storage of the cDNA preparation is allowed at minus 20 ^{o}C for not longer than one month.

8. PCR PROTOCOL

8.1 Mark tubes with PCR-mix for each test sample, negative control (C-), positive control (C+) and two tubes for background buffer (applicable to FLASH PCR kits).

For example if you need to test 10 samples, mark 12 tubes (10 for each sample, 1 for C-, 1 for C+). For FLASH PCR kit mark 14 tubes (10 for each sample, 1 for C-, 1 for C+ and 2 for background buffer).

Mark only the caps of the tubes when using Rotor-Gene Thermal Cycler.

8.2 Thaw PCR-buffer at the room temperature.

8.3 Mix the PCR-buffer and TECHNO Taq-polymerase thoroughly (3-5 sec), then spin briefly (1-3 sec) at room temperature (18–25 °C).

Hold TECHNO Taq-polimerase at room temperature as short time as possible. The overheating is detrimental to its performance.

8.4 Prepare the mixture of PCR-buffer and TECHNO Taq-polymerase (Taq-polymerase solution). Add into the one tube:

- 10 x (N+1) µL of PCR-buffer,
- 0,5 × (N+1) µL of TECHNO Taq-polymerase,

N — number of the marked tubes including C-, C+, background tubes.

For example if you need to test 10 samples (12 marked tubes), prepare mixture of PCR-buffer and TECHNO Taq-polymerase for 13 (12+1) tubes: 130 µL PCR-buffer + 6,5 µL TECHNO Taq-polymerase.

8.5 Vortex the tube with Taq-polymerase solution for 3-5 seconds and spin down the drops for 1-3 seconds at room temperature (18–25 °C). The maximum storage time for Taq-polymerase solution is 1 hour.

8.6 Add 10 µl of Taq-polymerase solution into each tube (except background tubes). Add 10 µl of background buffer into corresponding tubes (applicable to FLASH PCR kits). Avoid paraffin layer break.

8.7 Add one drop (~20 µl) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close tubes tightly.

8.8 Vortex the tubes with samples for 3-5 seconds and spin down the drops for 1-3 seconds.

8.9 Add 5,0 µl of DNA sample into corresponding tube. Avoid paraffin layer break. Do not add DNA into the C-, C+ and background (applicable to FLASH PCR kits) tubes. Avoid paraffin layer break.

Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips.

8.10 Add 5,0 µl of C- which has passed NA isolation stage and reverse transcription reaction into C- and background (applicable to FLASH PCR kits) tubes. Add C+ into corresponding tube. Avoid paraffin layer break.

8.11 Spin tubes briefly (1-3 sec).

8.12 Set the tubes to the Thermal Cycler.

8.13 Launch the Thermal Cycler software and run PCR according to instructions supplied with device, considering 35 µl reaction mix volume. See tables 4-8 to refer the cycling program and table 9 to refer the detection channels (applicable to Real-Time PCR kits). Using Thercyc cycler you need to choose «Rapid active regulation» regulation algorithm. Amplification programs correspondence with reagent kits is shown in Table 4.

Table 4. The PCR program for Tercyc Conventional PCR Thermal Cycler

Step	For thermal cyclers with active regulation			Number of cycles
	Temperature	Time		
		min	sec	
1	94	5	0	1
2	94 58 64	0 0 0	10 25 15	50
3	10	…	…	Storage

Table 5. The PCR program for DT*lite* and DT*prime* Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	94	5	00	1		Cycle
2	94	0	10	50		Cycle
	58	0	25		v	
	64	0	15			
5	10	…	…	Storage		Storage

Table 6. The PCR program for iCycler iQ5 thermal cyclers (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	05:00	94,0	
2	50				
		1	00:10	94,0	
		2	00:25	58,0	Real Time
		3	00:15	64,0	
3	1				
		1	01:00	10,0	

Table 7. The PCR program for iCycler iQ thermal cyclers (Bio-Rad Laboratories)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	00:30	80,0	
		2	05:00	94,0	
2	10				
		1	00:20	94,0	
		2	00:20	58,0	
		3	00:10	64,0	
3	2				
		1	00:20	85,0	Real Time
PCR program					
4	40				
		1	00:10	94,0	
		2	00:10	58,0	
		3	00:30	58,0	Real Time
		4	00:20	64,0	
5		…	…	10,0	Storage

Table 8. The PCR program for Rotor-Gene Thermal Cyclers

Cycling	Temperature	Hold Time	Cycle Repeats
Cycling	80 °C	300 sec	1 time
Cycling 2	94 °C	10 sec	50 times
	54 °C	5 sec	
	58 °C*	25 sec	
	64 °C	20 sec	

* Take the measurement

Table 9. Detection channels

	Specific product	IC
DT*prime*, DT*lite* and IQ5	FAM	HEX
Rotor-Gene	Green	Yellow

9. CONTROLS

Table 11.

Control	The controlled step	Result		Interpretation
		Specific signal is present	Specific signal is absent	
C+	PCR	+	+	Valid
		-	-	Invalid
C-	PCR and DNA extraction	+	+	Invalid
		-	-	Valid
RNA-IC	PCR and DNA extraction	+	+	Valid
		-		Valid
			-	Invalid

The sample is considered positive if the signal for specific DNA is present. The signal for IC could be absent in samples with high concentration of specific DNA due to competitive priming.

The sample is considered negative if the signal for specific DNA is absent and for RNA-IC is present.

If the signal for C- is present, whole tests of current batch considered false. Decontamination required.

10. DATA ANALYSIS

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers or Fluorescence Readers the analysis performed automatically. In all other cases the analysis is based on the presence or absence of specific signal. The controls should be also considered to exclude false positive and false negative results (see p. 7 of the current manual). The cutoff Ct values for Rotor-Gene thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

The analysis performed automatically. After completion of the run the device will build standard curve, define the concentration of viral DNA and form the report. The PCR efficiency should be in 90-100% range.

The interpretation should be performed in accordance with table 12.

Table 12.

HIV FLASH PCR detection Kit	Test samples		Interpretation
	HIV REAL-TIME PCR detection Kit		
	Fam	Hex	
"+"	Cp/Ct is specified	Not considered	Positive
"-"	Cp not specified (for iQ N/A)	Cp (for iQ5 Ct) 29-34	Negative
"uncertain"	Cp not specified (for iQ N/A)	Cp not specified (for iQ5 N/A)	uncertain
C+			
"+"	Cp is specified	Not considered	Positive
C-			
"-"	Cp not specified (for iQ N/A)	Cp 29-34 (for iQ5 Ct 29-34)	Negative

11. TROUBLESHOOTING

Table 13.

	Specific signal +	Specific signal -	Possible cause	Solution
C+	-	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	+	Contamination	Dispose current batch Perform decontamination procedures
RNA-IC		-	PCR inhibition	Repeat whole test

If you face to any undescribed issues contact our representative

12. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 9 month from the date of production.

All components of the **HIV PCR detection Kit** except PCR-mix and C+ must be stored at minus 20 °C over the storage period. The PCR-buffer and mineral oil can be stored at at 2-8 °C.

The PCR-mix, C+ and *PREP-NA* DNA/RNA Extraction Kit must be stored at 2-8 °C over the storage period.

Transportation can be held by all types of roofed transport with adherence to above mentioned temperature requirements.

An expired **HIV PCR detection Kit** must not be used.

We strongly recommend following the instructions to get robust and reliable results.

The conformity of the **HIV PCR detection Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our customer service by quality issues of the **HIV PCR detection Kit**: 115587, Moscow, Varshavskoye sh. 125g building 6, DNA Technology, LLC. Phone/Fax: +7(495)9804555 e-mail: help@dna-technology.ru, www.dna-technology.ru

13. SPECIFICATIONS

a. **Analytical specificity:** the **HIV PCR detection Kit** allows detection of HIV-1. The samples containing HIV-1 will be defined as positive. The samples not containing HIV-1 will be defined as negative.

b. **Sensitivity:** not less than 200 copies of HIV DNA per 1 mL of blood plasma.

c. **Diagnostic sensivity:** 99,5**%**.

d. **Diagnostic specificity:** 100**%.**

The claimed specifications are guaranteed when DNA extraction is performed with *PREP-NA* DNA/RNA Extraction Kit.

14. QUALITY CONTROL

"DNA-Technology, Research&Production" LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for *In Vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO ISO 9001:2008 and ISO 13485:2003

15. KEY TO SYMBOLS

Authorized representative in EU

Upper limit of temperature

Caution

Manufacturer

Consult instructions for use

Negative control

Date of manufacture

Positive control

Expiration date

Cataloque number

In vitro diagnostic medical device

Sufficient for

Batch code

Temperature limitation

Version

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